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# SELENIUM AND DRUG METABOLISM—III

## RELATION OF GLUTATHIONE-PEROXIDASE AND OTHER HEPATIC ENZYME MODULATIONS TO DIETARY SUPPLEMENTS

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**Abstract**—Male mice were fed a torula yeast-based diet containing different amounts of added selenium for a period of 4 months. Liver glutathione peroxidase activity assayed with $H_2O_2$ showed a logarithmic dependence on dietary selenium with a saturation plateau above 2 ppm Se and an extrapolated zero of 0.02 ppm Se. In contrast, liver selenium content and GSH-Peroxidase activity showed a linear correlation. Glutathione peroxidase activity became undetectable at a liver Se content of about 90 ng Se/g liver wet wt. Thus, about 10% of liver selenium is not related to GSH-Px activity. Five dietary groups were supplemented, respectively, with 0, 0.05, 0.5, 5.0 and 10 ppm Se in the form of $Na_2SeO_3$. Some changes in drug metabolism enzymes were observed with the high Se diets. An increase occurred in Non-Se-GSH activity as well as in ethacrynic acid-assayed GSH transferase, these are interpreted as early signs of Se toxicity. The diet containing 0.01 ppm Se with no supplementary Se produced the multiple hepatic enzyme modulations which were previously reported. The animals raised on this very low Se diet had normal hepatic contents of glutathione, α-tocopherol, calcium, magnesium, iron, zinc, copper and manganese compared to controls supplemented with 0.5 ppm Se. However, significant changes in the microsomal fatty acid pattern were observed while the total phospholipid content as well as membrane fluidity showed no differences between the two dietary groups.

Most of the interest in the biochemical background of the essential trace element selenium concentrated in recent years on the function and properties of selenium-containing GSH-peroxidase [1]. Unlike in bacteria, where several different selenoproteins have been identified and characterized [2], animal metabolic functions of selenium different from GSH-Px are in a very early stage of recognition. In the two preceding papers of this series [3, 4] we reported that a variety of hepatic enzyme modulations related to both phases of drug metabolism undergo specific and reversible modulations in severe Se deficiency in mice. We showed that during depletion these enzyme activity modulations occur in a synchronized way to reach an apparently final state after several months. On the other hand, a single pulse repletion of Se restored the modulations within a few days with distinct recovery kinetics for each enzyme. Finally, the concentration dependence of this repletion allowed its dissociation from GSH-Px. It required only 7 μg Se/kg for half-maximal restoration and GSH-Px required 250 μg Se/kg.

We have extended this investigation by studying the effect of supra-physiological amounts of dietary selenium on GSH-Px, liver selenium content and degree of drug metabolism enzyme modulations. Furthermore, we tried to exclude a possible influence on other trace elements, related cofactors and fluidity of the microsomal membrane in the animals that exhibited these Se-dependent metabolic disturbances.

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### MATERIALS AND METHODS

Weanling male albino mice (strain NMRI han) from the Zentralinstitut für Versuchtiere, Hannover, West Germany, were housed in plastic cages in groups of 6–8 animals with free access to food and water. They were fed a torula yeast-based diet as described [4]. The diet contained a basic selenium content of 0.01 ± 0.002 ppm Se (assayed fluorimetrically as well as by atomic absorption). Selenium supplements were added as $Na_2SeO_3$. The diet containing 0.5 ppm Se is referred to as control diet. For determination of other trace elements, lyophilyzed liver samples were wet ashed in the presence of $H_2O_2$ and concentrated $HNO_3$ in Teflon vessels. Copper, zinc, iron and manganese were then determined by atomic absorption spectrophotometry and calcium and magnesium by flame ionization spectrometry. Microsomes were extracted by the Folch procedure [5] in the presence of heptadecanoic acid methylester as internal standard and 0.05% butylhydroxy toluene as antioxidant. The extract was hydrolyzed in methanolic KOH and esterified in the presence of 7% boron trifluoride [6]. The methylesters were dried by $Na_2SO_4$ and 1 μl was injected into a Carlo–Erba 2150 gas chromatograph equipped with a 25 m long OV 110 capillary column. A temperature program from 30 to 240° was run within 40 min with a split of 1:100 using $H_2$ as carrier gas. The peaks were detected by flame ionisation. All other enzyme assays as well as the sources of chemicals and standards are described in the preceding paper [4]. Fluidity of microsomal membranes was measured as described

in [7] using diphenylhexatriene as a fluorescent probe.

## RESULTS

Table 1 contains data of several sets of experiments where mice were fed a selenium-deficient diet supplemented with 0 (extremely low), 0.05 (low) 0.5 (adequate), 5 (high) and 10 (very high) ppm selenium for a period of 4 months. A continuous response to any amount of supplement was only observed for GSH-Px activity in liver. No other parameter out of the 21 that were measured exhibited a significant difference between the "normal" 0.5 ppm diet and either the "low" 0.05 ppm or "high" 5 ppm selenium diet. Among the animals raised on 10 ppm Se, a decrease in DCNB-reactive GSH-transferase activity was observed, concomitant with no change in CDNB-reactive enzyme activity and about 100% increase of activity with ethacrynic acid as substrate. These results indicate that high doses of selenium affect different GSH-S-transferases in mouse liver in different ways. The observation that the Non-Se dependent GSH-Px was increased by the same factor as the ethacrynic acid reactive transferase might not be pure coincidence but rather suggests that this type of transferase may be responsible in mouse liver for Non-Se-GSH-Px.

When the adequate diet is compared with the two lower Se diets, it is evident that the 0.05 ppm diet contained too little Se to maintain GSH-Px activity but still too much to cause the other enzyme modulations. These became manifest only in the diet with no supplementation which had an intrinsic content of 0.01 ppm Se. After these experiments, we fed a commercially available semi-synthetic low-Se diet which contained $0.021 \pm 0.007$ ppm Se for 12 months. Animals fed this diet showed reduction of GSH-Px activities to 10% of control and a significant increase of DCNB-reactive GSH transferase to 120% but no other significant alteration in their liver enzyme pattern. This means that this diet was near the threshold in the trace element content with respect to enzyme modulation effects.

Figure 1 shows a correlation between hepatic GSH-Px activity in the mice fed the five different diets on the one hand and the selenium content of the livers on the other hand. A logarithmic relationship was found for the pair GSH-Px and Se-supplement. From 5 to 10 ppm dietary Se no further increase in the enzyme's activity seemed to occur. Extrapolation of the correlation line to zero led to a dietary Se content of 0.02 ppm below which no GSH-Px would be detectable. The GSH-Px activity correlated linearly with the Se content of the liver when the organ contained more than about 90 ng Se per gram wet wt. Below this content, which is equivalent to about 10% of the total liver Se, no correlation was evident. This observation suggests that this small part of Se might be involved in metabolic functions other than GSH-Px, presumably in processes related to the enzyme modulations observed in this very low supplementation range.

In order to further exclude possible secondary

Table 1. Specific activities of liver enzymes and cytochromes of mice that had been fed five differently supplemented torula yeast diets for 6 months. The basal diet contained $10 \pm 3$ ng/g selenium

| | Dietary selenium supplement | | | | |
|---|---|---|---|---|---|
| | 0 ppm | 0.05 ppm | 0.5 ppm | 5.0 ppm | 10.0 ppm |
| Cytochrome content: | | | | | |
| Cytochrome P-450 | $0.83 \pm 0.1$ | $0.79 \pm 0.03$ | $0.76 \pm 0.1$ | $0.79 \pm 0.2$ | $0.57 \pm 0.2^{\beta}$ |
| Cytochrome $b_5$ | $0.35 \pm 0.02$ | $0.38 \pm 0.03$ | $0.43 \pm 0.07$ | $0.41 \pm 0.03$ | $0.30 \pm 0.09^{\beta}$ |
| Enzyme activities: | | | | | |
| Ethoxycoumarine deethylase[b] | $4.4 \pm 1.4^{\gamma}$ | $1.6 \pm 0.4$ | $1.3 \pm 0.4$ | $1.4 \pm 0.1$ | $1.2 \pm 0.2$ |
| Aminopyrine-*N*-demethylase[b] | $6.8 \pm 1.1^{\alpha}$ | $6.4 \pm 0.6$ | $7.6 \pm 0.7$ | $7.2 \pm 0.7$ | $7.9 \pm 0.9$ |
| Aniline hydroxylase[b] | $0.5 \pm 0.1$ | $0.6 \pm 0.1$ | $0.6 \pm 0.04$ | $0.5 \pm 0.2$ | $0.5 \pm 0.1$ |
| Cytochrome P-450-oxidase[b] | $5.1 \pm 0.9$ | $4.8 \pm 0.7$ | $4.6 \pm 0.8$ | $4.8 \pm 0.6$ | $5.2 \pm 1.2$ |
| Cytochrome P-450-peroxidase[b] | $109 \pm 18^{\gamma}$ | $57 \pm 10$ | $59 \pm 7$ | $53 \pm 10$ | $61 \pm 13$ |
| NADPH-cytochrome-c-reductase[c] | $81 \pm 5^{\gamma}$ | $113 \pm 5$ | $124 \pm 24$ | $148 \pm 17$ | $122 \pm 12$ |
| NADH-cytochrome-c-reductase[b] | $660 \pm 53$ | $780 \pm 43$ | $700 \pm 108$ | $840 \pm 150$ | $825 \pm 130$ |
| Flavin-containing monooxygenase[b] | | | | | |
| *N*-oxide formation | $2.7 \pm 0.4^{\gamma}$ | $4.1 \pm 0.3$ | $4.4 \pm 0.5$ | $4.1 \pm 0.4$ | $4.6 \pm 0.8$ |
| *N*-demethylation | $0.9 \pm 0.1^{\gamma}$ | $1.6 \pm 0.3$ | $1.9 \pm 0.4$ | $1.7 \pm 0.4$ | $1.7 \pm 0.3$ |
| UDP-glucuronyltransferase[b] | $3 \pm 0.8^{\gamma}$ | $1.1 \pm 0.3$ | $1.4 \pm 0.2$ | $1.3 \pm 0.2$ | $1.5 \pm 0.2$ |
| Sulfotransferase | $0.13 \pm 0.03$ | $0.22 \pm 0.03$ | $0.24 \pm 0.01$ | $0.22 \pm 0.02$ | $0.21 \pm 0.03$ |
| GSH-S-transferases | | | | | |
| 1.2-Dichloro-4-nitrobenzene[c] | $86 \pm 19^{\gamma}$ | $28 \pm 9$ | $28 \pm 9$ | $28 \pm 7$ | $22 \pm 8^{\beta}$ |
| 1-Chloro-2,4-dinitrobenzene[d] | $3.6 \pm 0.3^{\gamma}$ | $1.7 \pm 0.4$ | $1.5 \pm 0.3$ | $1.5 \pm 0.1$ | $1.7 \pm 0.5$ |
| Ethacrynic acid [c] | $123 \pm 10$ | $128 \pm 22$ | $120 \pm 16$ | $129 \pm 9$ | $241 \pm 19^{\gamma}$ |
| Se-GSH-Px[c] | $8.8 \pm 2.8^{\gamma}$ | $113 \pm 20^{\gamma}$ | $690 \pm 110$ | $1020 \pm 110^{\gamma}$ | $1150 \pm 150^{\gamma}$ |
| Non-Se-GSH-Px[c] | $330 \pm 60^{\gamma}$ | $290 \pm 45$ | $330 \pm 53$ | $490 \pm 210$ | $950 \pm 260^{\gamma}$ |
| GSSG-reductase[c] | $350 \pm 37^{\gamma}$ | $190 \pm 13$ | $200 \pm 19$ | $200 \pm 30$ | $200 \pm 7$ |
| GSH-thioltransferase[c] | $9 \pm 1^{\gamma}$ | $14 \pm 3$ | $14 \pm 2$ | $14 \pm 2$ | $15 \pm 2$ |
| Hemeoxygenase[b] | $0.13 \pm 0.04^{\gamma}$ | $0.07 \pm 0.02$ | $0.06 \pm 0.01$ | $0.07 \pm 0.01$ | $0.08 \pm 0.02$ |

Data are given in means ± S.D., N = 6. [a] nmole/mg [b] nmole/mg min [c] mU/mg [d] U/mg. Significances (with reference to the 0.5 ppm Se group): $\alpha$: $P \leq 0.05$; $\beta$: $P \leq 0.01$; $\gamma$: $P \leq 0.005$ (Student's *t*-test).

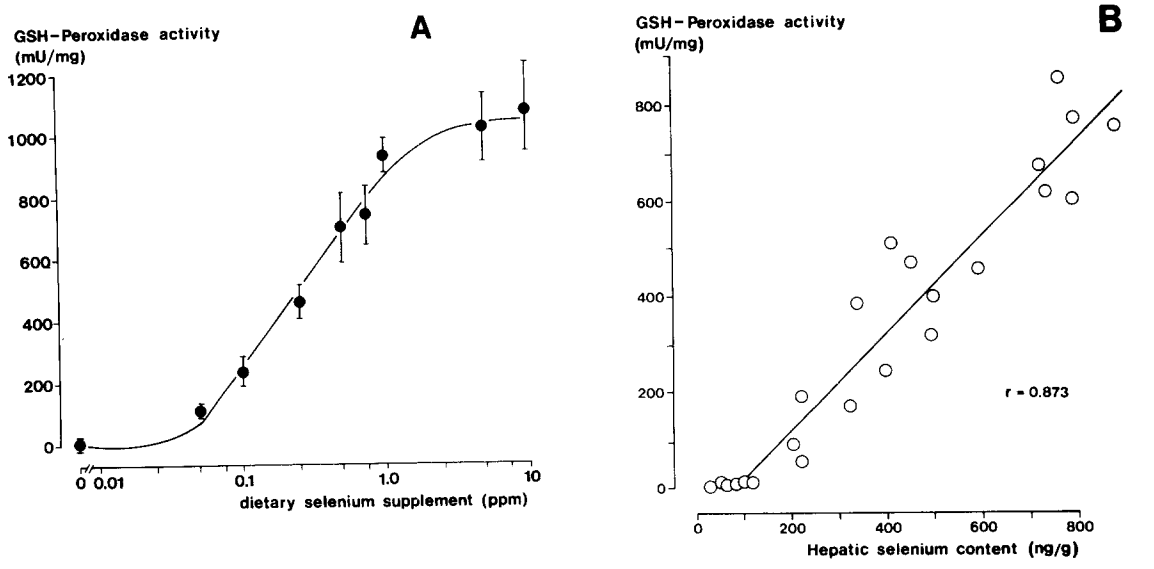


Fig. 1. Relationship between liver GSH peroxidase activity assayed with $H_2O_2$ as substrate and dietary selenium supplement in the form of sodium selenite in mice (A) and liver selenium content (B) after a feeding period of 6 months.

effects exerted under these nutritional conditions by the lack of GSH-Px, we investigated the phospholipid content as well as the fatty acid content of the microsomal fraction of our animals. Table 2 illustrates that changes in the content of individual fatty acids became evident in severely Se-deficient mice, while the total phospholipid content showed no significant differences among the non-supplemented and the control group. Surprisingly, the most prominent change was a 30% increase in arachidonic acid, accompanied by a decrease in docosahexaenoic acid and oleic acid. However, when the sum of the degree of unsaturation was calculated for the fatty acids in both groups, identical values were obtained. We also measured the membrane fluidity of the microsomes of both groups by the fluorescence polarization method using diphenylhexatriene as a probe. No significant differences were observed in the fluidity parameters between the two groups.

Finally, we assessed six essential trace elements as well as $\alpha$-tocopherol and GSH contents of our animals' livers in order to exclude deprivation of another micronutritient during the long feeding period on the highly artificial diet. The results shown in Table 3 demonstrate that none of these substances seems to be affected.

## DISCUSSION

This study shows that over a wide range of dietary Se supplementation drug metabolizing and related enzymes of mouse liver remain unaffected except Se-GSH-Px. At potentially toxic Se intakes, some GSH-transferase activities fall while the transferase activity assayed with either ethacrynic acid or with cumenehydroperoxide plus GSH increases considerably. These phenomena could be useful for diagnostic purposes because they, as well as liver GSH-Px, yield information about the selenium supply of the animal. However, below a supplement of 0.02 ppb or a liver content of about 90 ng Se/g, different conditions apply: this small residual pool

Table 2. Phospholipid content and fatty acid composition of liver microsomes from control mice (0.5 ppm Se supplement) and animals that had been fed a non-supplemented torula-yeast diet for 6 months

| | | $Se^+$ | $Se^-$ |
|---|---|---|---|
| Phospholipid contents ($\mu$moles/mg) | | 0.44 ± 0.07 | 0.49 ± 0.12 |
| Fatty acids: | | | |
| Palmitic | (16:0) | 19.8 ± 1.4 | 20.5 ± 2.0 |
| Palmitoleic | (16:1) | 1.7 ± 0.1 | 1.7 ± 0.3 |
| Stearic | (18:0) | 15.5 ± 1.3 | 15.0 ± 1.0 |
| Oleic | (18:1) | 15.0 ± 0.9 | 13.2 ± 0.6$^{\beta}$ |
| Linoleic | (18:2) | 14.6 ± 0.7 | 15.8 ± 1.2$^{\alpha}$ |
| Arachidonic | (20:4) | 14.0 ± 0.6 | 18.5 ± 1.1$^{\gamma}$ |
| Docosahexaenoic | (20:6) | 18.0 ± 2.0 | 15.2 ± 0.6$^{\beta}$ |

Data in means ± S.D., N = 6. Significances: $\alpha$: $P \leq 0.05$; $\beta$: $P \leq 0.01$; $\gamma$: $P \leq 0.005$ (Student's $t$-test).

Table 3. Cofactor and trace element content of control liver and organs from animals that had been fed a non-supplemented torula-yeast diet for 6 months. Controls were supplemented with 0.5 ppm Se

| | $Se^-$ | $Se^+$ |
|---|---|---|
| Liver protein[a] | 164 ± 26 | 173 ± 30 |
| Glutathione | 18 ± 2 | 19 ± 2 |
| α-Tocopherol | 0.13 ± 0.06 | 0.13 ± 0.04 |
| Calcium | 6.8 ± 1 | 6.85 ± 2 |
| Magnesium | 42 ± 2 | 45 ± 4 |
| Iron | 4.4 ± 3 | 6.9 ± 2 |
| Zinc | 2.8 ± 0.4 | 2.8 ± 0.4 |
| Copper | 0.4 ± 0.1 | 0.3 ± 0.1 |
| Manganese | 0.16 ± 0.05 | 0.13 ± 0.06 |
| Selenium | 0.005 ± 0.002[δ] | 0.067 ± 0.005 |

Data in means ± S.D. (nmoles per mg liver protein). δ: $P \leq 0.001$; $N = 6$ (Student's $t$-test).
[a] mg per gram wet wt.

of Se seems not to be available for GSH-Px. Although we do not know the chemical form or the availability of this Se fraction we assume that it plays an important role in a series of events that lead to multiple metabolic disturbances in various compartments of the liver cell. From the uniformity of the repletion dose-response we previously concluded that a centrally located regulatory process might be affected when this part of the Se pool is also emptied. Several control experiments reported here suggest that neither other micronutrients nor secondary membrane alterations could be responsible for these effects. In contrast, the constant degree of unsaturation of membrane lipids and their similar fluidity rather lead to a conclusion that the fatty acid changes observed might be the result of a counter-regulation.

Another point deserves special mention, i.e. the residual Se in our basal diet. Since we do not know the form of Se or its bioavailability, we are unable to differentiate between the effect of this remainder and an actual zero supplementation. However, we believe that we compared a diet containing organic Se of low bioavailability.

In addition this study stresses the need for precise characterization of the state of selenium deficiency in animals. Obviously, a drop of GSH-Px by e.g. 90% in an organ is not sufficient to allow characterization of these animals as severely Se-deficient. Many inconsistent results in the past might be related to this circumstance. In our particular case it now seems important to investigate whether the severe deficiency effects observed here are exclusively restricted to drug metabolism. Other pathways of metabolism may be affected as well.

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## REFERENCES

1. L. Flohe, in *Free Radicals in Biology V* (Ed. W. Pryor), p. 223. Academic Press, New York (1982).
2. Th. Stadtman, *Fund. appl. Toxicol.* **3**, 420 (1983).
3. R. Reiter and A. Wendel, *Biochem. Pharmac.* **33**, 3063 (1983).
4. R. Reiter and A. Wendel, *Biochem. Pharmac.* **33**, 1923 (1984).
5. J. Folch, M. Lees and G. H. Sloane-Stanley, *J. biol. Chem.* **226**, 497 (1964).
6. R. M. C. Dawson, N. Hemmington and J. B. Davenport, *Biochem. J.* **84**, 497 (1962).
7. R. Stocker, K. H. Winterhalter and Ch. Richter, *FEBS Lett.* **144**, 199 (1982).